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IMPROVED DIAGNOSIS TOOLS FOR THE DETECTION OF YAM VIRUS IN THE SANITATION PROCESS AND UNVEIL VIRUS-FREE ACCESSIONS FOR PRODUCERS' EXCHANGE

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Mots clés: Yams, viruses, sanitation, viral diagnosis.

Abstract

French West Indies Biological Resources Centre for Tropical Plants (CRB-PT) maintains several germplasm collections of tropical crops and wild relatives, including a collection of more than 450 yam accessions (*Dioscorea spp*) in vitroculture. The purpose of this Centre is to conserve this biodiversity and distribute virus-free germplasm to end users.

To this aim, virus populations infecting conserved accessions have been characterized and the diversity of intra and inter-species highlighted through the SafePGR project. Thus, three new virus generi have been found out (Ampelovirus, Macluravirus and Sadwavirus) besides those existing (Badnavirus [1], Potyvirus [2] and Potexvirus [3]). Efficient extraction method and appropriate detection tools have been created and/or optimized, then implemented for an initial diagnosis of the *in vitro* collection. Prevalence shown that more than 75% of yam accessions are infected by Potyvirus, 80% of *D. alata*, the most cultivated yam, are infected by the new yam virus Ampelovirus and Macluravirus are only present in *D. alata*.

Only 14 accessions remained free of viruses, but the majority of the collection contained one, two or more of viruses, so the necessity to sanitize the infected germplasm. The sanitation process consists to submit shoots to thermotherapy at 34°C, then in excising apical meristem to obtain the regeneration of a new plant, expecting to be free of viruses. Using optimized detection tools, each new plant is diagnosed for the six viral generi.

Up to now, the sanitation process leads to the achievement of 8 accessions completely virus-free. The development and yield of this improved plant material have to be evaluated by comparison of infected and sanitized accessions.

Materials and methods

Through SafePGR project, successful, effective and sensitive techniques have been implemented to detect new and already known yam viruses. Total nucleic acids (tNAs) of high quality were first extracted with silica powder and all RNA viruses were detected from the same cDNA by RT-PCR method. Five viral generi, included 3 new ones (Ampelovirus, Macluravus and Sadwavirus), and 3 distinct species of Potyvirus (YMV and YMMV) and Potexvirus (YVX) were indexed. As Badnavirus is integrated in yam genome [4], this DNA virus was detected by using antibody in immunocapture-PCR. These new detection methods were tested on *in vitro* yam collection of CRB-PT to perform initial diagnosis of yam germplasm. Thus, 275accessions were indexed, including 73 *Dioscorea alata*, 47 *D. cayenensis-rotundata* and 150 *D. trifida*. Based on this work, a set of 15 accessions was chosen to undergo a cycle of sanitation consisting in thermotherapy and apical meristem culture. The viral status of each generated meriplant was checked with detection methods listed above.

Main results

The collection indexation showed that the *D. trifida*, species which is native to the central tropical America, is particularly sensitive to viral infections, as already known, with a high prevalence for Potyvirus (81%), Badnavirus (46%) and Potexvirus (22%) compared to other species. *D. alata* presents the highest rate of contamination by the Ampelovirus (80 %) and is also the only species infected by Macluravirus. The Sadwavirus prevalence is equivalent for all the yam species.

The sanitation process was performed on 46 *D. trifida*, 10 *D. alata* and 5 *D. cayenensis-rotundata* and we obtained 8 accessions completely virus-free, whatever their initial contamination. The rates of sanitation depend on the virus, for instance 16 % for YMMV (Potyvirus), 56% for YMV (Potyvirus) or 100 % for Macluravirus.

Conclusion

We succeed to implement the sanitation on *in vitro* yam collection of the CRB-PT thank to efficient and reliable viral detection methods. 8 accessions have already been sanitized but the efficiency strongly depends on the considered virus. Macluravirus, which infects only *D. alata* with a low prevalence, are removed quite easily (100% of sanitation) and the Potyvirus YMV, which leads the strongest loss of yield in yam, has fortunately a good sanitation rate (56%). Conversely, the Potyvirus YMMV, that infects 72% of yam collection, preferentially remains in plants with a sanitation rate of 16%. However, the sanitation rates could depend on yam species, but our sets of accessions for sanitation mainly contain the American species, *D. trifida*.

The sanitation of yam germplasm is a complicated and time-consuming process and several cycles are necessary to obtain a complete sanitation.

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